Association between neuropeptide Y receptor Y2 promoter variant rs6857715 and major depressive disorder

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Stress increases the risk for major depressive disorder (MDD), overeating, and alcohol dependence (AD). The neuropeptide Y system is one of the best-known modulators of the stress response, and some of its effects are mediated through the neuropeptide Y receptor Y2 (*NPY2R*). The functional *NPY2R* variant rs6857715 (C-599T) has been implicated in both obesity and AD, but with opposing alleles. The present study explored whether rs6857715 is also associated with MDD. Analysis of the overall sample (595 MDD cases; 1295 controls) showed an association with the AD risk allele C [*P* = 0.020, odds ratio (OR) (C-allele) = 1.18]. The association remained significant after excluding MDD patients with AD/alcohol abuse [*P* = 0.038, OR (C-allele) = 1.18]; increased weight/appetite [*P* = 0.006, OR (C-allele) = 1.23]; or both [*P* = 0.008, OR (C-allele) = 1.25]. The present findings suggest that the *NPY2R* rs6857715 C-allele makes a genuine contribution toward MDD. *Psychiatr Genet* 27:34–37 Copyright © 2017 Wolters Kluwer Health, Inc. All rights reserved.

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Introduction

Stress increases the risk for major depressive disorder (MDD), alcohol dependence (AD), and obesity (Kendler *et al.*, 1999; Sinha and Jastreboff, 2013). These conditions manifest in both isolated and comorbid states. Here, epidemiological studies have reported comorbidity between MDD and AD (Petrakis *et al.*, 2002; Hasin *et al.*, 2005) and between MDD and obesity (Petry *et al.*, 2008; de Wit *et al.*, 2010). In humans and animals, one of the most widely reported mediators of resilience to stress is neuropeptide Y (NPY) (Hirsch and Zukowska, 2012; Sah and Geracioti, 2013). NPY exerts some of its effects through the neuropeptide Y receptor Y2 (*NPY2R*). Genetic variation in *NPY2R* may thus determine individual response to stress and the propensity to stress-induced disorders (Enman *et al.*, 2015). Genetic studies have reported an association between a functional variant in the *NPY2R* promoter (rs6857715; synonym: C-599T; higher expression reported for the rs6857715 T-allele (Wei *et al.*, 2013)) and both obesity (Siddiq *et al.*, 2007) and AD [Frank *et al.*, 2012 (rs6857715 *P* = 0.005, odds ratio (OR) (C-allele) = 1.16); Wetherill *et al.*, 2008]. For further information, see Supplementary Text, Supplemental digital content 1 (*http://links.lww.com/PG/A167*) and Supplementary figs S2, Supplemental digital content 1 (*http://links.lww.com/PG/A167*); S3, Supplemental digital content 1 (*http://links.lww.com/PG/A167*); S4, Supplemental digital content 1 (*http://links.lww.com/PG/A167*); and S5, Supplemental digital content 1 (*http://links.lww.com/PG/A167*). However, risk was conferred by the T-allele in obesity and the C-allele in AD. To our knowledge, no candidate study to date has tested whether rs6857715 is also associated with MDD.

The aim of the present study were two-fold. First, we investigated whether the *NPY2R* variant rs6857715 is associated with MDD. Second, we investigated whether this association is genuine or instead because of subgroups of MDD patients with comorbid AD/alcohol

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abuse or increased appetite/weight. Given the previously reported association of the C-allele with AD and of the T-allele with obesity, we hypothesized that MDD patients with AD/alcohol abuse would show increased C-allele frequency, whereas MDD patients with increased appetite/weight would show increased T-allele frequency in comparison with MDD patients without these comorbid conditions.

Materials and methods

Participants

The present German sample comprised 595 MDD patients and 1295 population-based controls. Data on AD/alcohol abuse were available for 537 MDD patients and 56 MDD patients showed one of these disorders. Data on appetite increase and/or weight gain were available for 594 MDD patients and 84 MDD patients showed one of these features. Thirteen MDD patients showed both AD/alcohol abuse and increased weight/appetite.

In patients, clinical symptoms and lifetime 'best estimate' diagnoses of MDD, AD, and alcohol abuse were assessed using multiple sources of information including the Structured Clinical Interview for *Diagnostic and Statistical Manual of Mental Disorders*, 4th ed. (DSM-IV) Axis I Disorders (First *et al.*, 1994); medical records; and information obtained using the family history method. The items 'appetite increase' and 'weight gain' were assessed using the Structured Clinical Interview for DSM-IV Axis I Disorders and patients were asked to indicate whether or not they considered these changes to have been secondary to medication. The items were only rated as present in individuals for whom appetite increase and/or weight gain could be attributed to the diagnosis per se and not to medication. Diagnosis was assigned according to the DSM-IV criteria. In each case, a consensus diagnosis was assigned by two raters. All patients had a history of at least one episode of MDD severe enough to warrant hospitalization.

The study was approved by the respective local ethics committees and all participants provided written informed consent before inclusion.

Single nucleotide polymorphism selection and genotyping

The NPY2R promoter variant rs6857715 was selected as it is reported to confer allele-specific expression differences *in vitro* (Wei *et al.*, 2013). In addition, rs6857715 has been associated with AD and obesity (Siddiq *et al.*, 2007; Wetherill *et al.*, 2008; Frank *et al.*, 2012; Supplementary Text, Supplemental digital content 1, *http://links.lww.com/PG/A167* and Supplementary fig. S5, Supplemental digital content 1, *http://links.lww.com/PG/A167*). Detailed information on genotyping is provided in Rietschel *et al.* (2010). Experimental genotyping cluster plots for rs6857715 are shown in Supplementary fig. S1, Supplemental digital content 1 (*http://links.lww.com/PG/A167*).

Statistical analysis

Power calculations were performed using the Genetic Power Calculator (Purcell *et al.*, 2003; *http://pngu.mgh.harvard.edu/~purcell/gpc/cc2.html*) using a multiplicative model of inheritance; a disease/marker allele frequency of 0.61 (1000 Genomes CEU reference sample; *http://www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?rs=6857715*); a lifetime prevalence of major depressive episodes in Germany of around 10% (Kessler and Bromet, 2013); a control : case ratio of 2.18 ($D'=1$) between disease and marker allele; and a significance level of $\alpha=0.05$. The power of the sample to detect association with MDD ranged from 53 to more than 80% depending on the proposed effect size. For a proposed effect size of OR (C-allele) = 1.16, as based on the association with AD (Frank *et al.*, 2012), the power was 53%. For a proposed effect size of OR (T-allele) = 1.345, as based on the association with obesity (Siddiq *et al.*, 2007), the power was 98%. In Siddiq *et al.* (2007), the OR was calculated from the genotype counts of 204/208/67 obese adults and 475/378/85 controls. The minimum effect size detectable in the present sample is OR = 1.23.

Deviation from Hardy–Weinberg equilibrium (HWE) was calculated using an exact test (*http://ihg.gsf.de/cgi-bin/hw/hwa1.pl*). Association testing for rs6857715 was performed using the Armitage Trend Test (*http://ihg.gsf.de/cgi-bin/hw/hwa1.pl*).

Results

Genotype counts, allele frequencies, and deviation from HWE results are shown in Table 1 (Supplementary fig. S4, Supplemental digital content 1, *http://links.lww.com/PG/A167*). Distribution of genotypes did not deviate from HWE (Table 1).

Comparison of all MDD patients ($N=595$) versus controls ($N=1295$) showed a significant association between MDD and the C-allele of rs6857715 [$P=0.020$, OR (C-allele) = 1.18]. This association remained significant when the analysis was restricted to MDD patients without AD/alcohol abuse versus controls [$P=0.038$, OR (C-allele) = 1.18]; MDD patients without increased weight/appetite versus controls [$P=0.006$, OR (C-allele) = 1.23]; and MDD patients with neither AD/alcohol abuse nor increased weight/appetite versus controls [$P=0.008$, OR (C-allele) = 1.25]. No significant association was found in a case-only analysis of MDD patients without AD/alcohol abuse versus MDD patients with AD/alcohol abuse [P (one sided) = 0.302, OR (C-allele) = 1.11]. Comparison of MDD patients without increased weight/appetite versus MDD patients with increased weight/appetite almost reached significance [P (one sided) = 0.054, OR (T-allele) = 1.31] (Table 2).

Table 1 Number of individuals per group, rs6857715 genotype counts, allele frequencies, and deviation from Hardy–Weinberg equilibrium

Group/subgroup	Number of individuals	Genotype counts (CC, CT, TT)	Allele frequency (C)	HWE[c]
MDD patients	595	240, 279, 76	0.64	0.79
MDD patients with appetite increase/weight gain[a]	84	30, 38, 16	0.58	0.51
MDD patients without appetite increase/weight gain[a]	510	210, 240, 60	0.65	0.56
MDD patients with AD/alcohol abuse[b]	56	25, 24, 7	0.66	0.77
MDD patients without AD/alcohol abuse[b]	481	191, 230, 60	0.64	0.49
MDD patients without appetite increase/weight gain and without AD/alcohol abuse (MDD only)	414	171, 196, 47	0.65	0.45
Population-based controls	1295	465, 619, 211	0.60	0.86

AD, alcohol dependence; HWE, Hardy–Weinberg equilibrium; MDD, major depressive disorder.
[a]Data were available for 594 of the 595 patients.
[b]Data were available for 537 of the 595 patients.
[c]HWE exact test *P*-values (according to *http://ihg.gsf.de/cgi-bin/hw/hwa1.pl*).

Table 2 *P*-values and effect sizes

Comparison groups/subgroups	*P*-value[a]	Effect size[b]
MDD patients versus population-based controls	$P = 0.020$ (two sided)	OR (C-allele) = 1.18 CI = 1.03–1.36
MDD patients without appetite increase/weight gain versus population-based controls	$P = 0.006$ (two sided)	OR (C-allele) = 1.23 CI = 1.06–1.43
MDD patients without AD/alcohol abuse versus population-based controls	$P = 0.038$ (two sided)	OR (C-allele) = 1.18 CI = 1.01–1.37
MDD patients without appetite increase/weight gain and without AD/alcohol abuse versus population-based controls (MDD only)	$P = 0.008$ (two sided)	OR (C-allele) = 1.25 CI = 1.06–1.47
MDD patients with appetite increase/weight gain versus MDD patients without appetite increase/weight gain	$P = 0.109$ (two sided) $P = 0.054$ (one sided)	OR (T-allele) = 1.31 CI = 0.94–1.83
MDD patients with AD/alcohol abuse versus MDD patients without AD/alcohol abuse	$P = 0.604$ (two sided) $P = 0.302$ (one sided)	OR (C-allele) = 1.11 CI = 0.74–1.68

AD, alcohol dependence; CI, confidence interval; MDD, major depressive disorder; OR, odds ratio.
[a]Armitage Trend Test *P*-values (according to *http://ihg.gsf.de/cgi-bin/hw/hwa1.pl*).
[b]Allelic OR and CI (according to *http://ihg.gsf.de/cgi-bin/hw/hwa1.pl*).

Discussion

The present analyses showed a significant association between MDD and the C-allele of the *NPY2R* promoter variant rs6857715, that is, the previously reported risk allele for AD. Exclusion of subgroups showed that this association is genuinely attributable to MDD and not to comorbid AD/alcohol abuse or increased weight/appetite. As hypothesized, the MDD patient group with increased weight/appetite showed a higher T-allele frequency than the MDD patient group without increased weight/appetite. This difference just failed to reach significance [P (one sided) = 0.054], which may have been attributable to the small sample size. However, if a large proportion of our MDD patients had had increased weight/appetite, the contrasting allelic effects on MDD and weight/appetite might have obscured the detection of any association.

A significant change in weight and/or appetite is a key diagnostic feature of MDD. Although significant loss of weight and/or decrease in appetite are typical features of so-called endogenous depression (Paykel, 2008), increased weight/appetite are reported by more than 40% of patients with atypical depression (Sullivan *et al.*, 1998; Thase, 2007). Research has shown that atypical depression is the most common form of depression in outpatient psychiatry (Nierenberg *et al.*, 1998; Singh and Williams, 2006). The term 'atypical' is used to distinguish this form of illness from the more severe 'endogenous' depression phenotype, which was the norm at the time when depression was rarely diagnosed in outpatients (American Psychiatric Association, 2013). The concept of endogenous versus reactive/atypical depression has been the subject of contentious debate for decades and has been abandoned because of lack of consistent biological support and therapeutic implications (Paykel, 2008). However, clearly measurable differences between patient subgroups, such as weight gain/loss, could facilitate dissection of clinical heterogeneity for the purposes of genetic studies.

As differences in the peptide NPY regulate both stress and food intake (Maniam and Morris, 2012), T-allele carriers may constitute a specific group of patients who are more likely to react to stress (e.g. stressful life events and/or MDD) by increased appetite/weight gain than is the case for C-allele carriers. Animal studies support this hypothesis as they have shown that stress upregulates *Npy2r* in a glucocorticoid-dependent manner and that upregulated *Npy2r* stimulates fat angiogenesis, leading to a 50% increase in visceral fat within a 2-week period. In contrast, *Npy2r* pharmacological inhibition or knockdown prevents the accumulation of fat under stress (Kuo *et al.*, 2007, 2008). The higher *NPY2R* expression reported for the T-allele (Wei *et al.*, 2013) is consistent with such a hypothesis. To investigate the role of the T-allele in atypical depression, large longitudinal studies of stress are warranted.

Conclusion

The present findings in MDD patients with a history of inpatient treatment support the hypothesis that the *NPY2R* variant rs6857715 is implicated not only in AD and obesity but also in MDD. Furthermore, our findings suggest that homogeneity in terms of MDD symptoms such as weight gain may facilitate genetic research into depression. In this context, it would be of interest to include information on appetite/weight increase in the Psychiatric Genomics Consortium MDD genome-wide association study analysis. In a previous mega-analysis, the Psychiatric Genomics Consortium included both inpatients and outpatients, and identified no association between this variant and the categorical diagnosis MDD ($P = 0.590$; Major Depressive Disorder Working Group of the Psychiatric GWAS Consortium *et al*., 2013; *http://www.med.unc.edu/pgc/downloads*). Further investigation of stress-related phenotypes may generate insights into the mechanisms acting across somatic and psychiatric diagnoses.

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Conflicts of interest

There are no conflicts of interest.

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